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VIII.

CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY, UNDER THE DIRECTION OF E. L. MARK, XLII.

ON THE CELL LINEAGE OF THE ASCIDIAN EGG.

A PRELIMINARY NOTICE.

By W. E. Castle.

Communicated by E. L. Mark, June 14, 1894.

The following paper is based on an uncompleted study of the early stages in the development of *Ciona intestinalis*, the material for which I was enabled to collect at the Newport Marine Laboratory in the summer of 1893, through the kindness of Mr. Alexander Agassiz.

Tunicate embryology has been studied from the standpoint of cell lineage to a greater or less extent, by Van Beneden et Julin ('84), Seeliger ('85), and Chabry ('87). The first named authors have followed the segmentation of the egg of *Clavelina Rissoana* as far as the 32-cell stage in a manner that admits of no question as to its accuracy. Every cell division is evidenced by karyokinetic figures that prove beyond doubt the actual genetic relationship.

The cell lineage of a single additional stage is given, one of forty-four cells. This stage must have been derived from the preceding by twelve cell divisions, six in each of the equivalent halves of the egg. Of these six, three are clearly indicated by spindles in the 32-cell stage. The other three were apparently determined by conjecture, and it is my purpose to show that by yielding themselves to conjecture in so small a matter as these three cell divisions, the eminent authors fell into an error which invalidates the most important conclusions of their otherwise excellent work. For in correlating the 44-cell stage with the 32-cell stage they have changed the orientation, so that they have identified the dorsal side of one with the ventral side of the other, the endodermal half of one with the ectodermal half of the other. *Their orientation of all the stages prior to the 44-cell stage* *is accordingly wrong. Their terms ectodermal and endodermal, ventral and dorsal, as employed up to this stage, must be interchanged.*

I shall in the present paper reproduce the figures of the 32-cell and 44-cell stages given by Van Beneden et Julin, with the proper orientation and the probable cell lineage as inferred from that actually determined in Ciona.

Chabry, in a paper concerned chiefly with teratology, traces the cell lineage of *Ascidiella aspersa* to a stage with thirty-two cells, and finds it identical, cell for cell, with that of Clavelina as given by Van Beneden et Julin. He however adopts, apparently without question, the orientation given by them, and makes accordingly, in his early stages the same error, calling dorsal (endodermal) that side of the egg which is really ventral (ectodermal).

He gives in part the cell lineage of the ectodermal side of a single older stage, in which five additional divisions are represented as having occurred in each of the equivalent halves of the egg. (See his Fig. 20.) These agree exactly with the divisions I have found occurring in Ciona subsequent to the 32-cell stage.

Seeliger studied an undetermined species of *Clavelina*, the same genus on which Van Beneden et Julin worked. It has been more than once observed by writers on tunicate embryology, that, though engaged with the study of forms so nearly related, these authors differ widely in their conclusions.

In discussing the 4-cell stage, for example, Van Beneden et Julin state that the two larger cells give rise to the anterior portion of the embryo, and in this I fully agree, whereas Seeliger contends that they produce the posterior portion. Seeliger however gives little evidence in support of his view, for his determination of the cell lineage is in most cases so manifestly a matter of mere conjecture that it hardly merits a serious consideration. But it may be worth while to point out where he has made the mistake that led to his false conclusion. It is in passing from the 16-cell stage to the next succeeding stage, the number of cells in which he did not take the pains to determine. In doing this he has reversed the poles anterior and posterior. For his 16-cell stage (his Figs. 14 and 15), *if its anterior and posterior poles be reversed*, corresponds unmistakably, both in the characteristic arrangement of its cells and in their relative size, with the 16-cell stage figured by Van Beneden et Julin (their Figs. 8 and 8a), and that figured by Chabry (his Figs. 17 and 24), and I can add from my own observations that it also corresponds with that of Ciona.

Seeliger's next stage (his Fig. 16) is oriented correctly, the posterior end being clearly indicated by the smaller marginal cells (left unshaded in his figure). This criterion for the posterior end of the embryo had been previously pointed out by Van Beneden et Julin, has since been recognized by Chabry and by Davidoff for the forms they studied, and holds good for Ciona also.

Seeliger speaks of his Figure 16 as representing a stage with fourteen "endoderm" cells (that is, cells which have been derived from the four dorsal cells of the 8-cell stage). It is highly probable that he should have represented as "endodermal" also a pair of lateral marginal cells left unshaded in his figure, thus bringing the number of descendants of the four dorsal cells of the 8-cell stage up to sixteen, the number we should expect as the result of two successive divisions in that hemisphere. The two cells in question correspond in position with the cells $c^{6.5}$ and $d^{6.5}$ of Figure 4 of this paper, except that in the egg of Ciona figured the cells named do not reach the margin, but lie a little more central.

Interpreted thus, Seeliger's Figure 16 corresponds closely with a similar view of the 32-cell stage of Van Beneden et Julin (reproduced in Figure 8 of this paper), and with Chabry's Figure 26, and with the same stage of Ciona (Figure 4 of this paper).

From this stage on, all investigators are agreed as to which is the anterior and which the posterior end of the embryo. If then the orientation of Seeliger's later stages is correct (and this will be conceded by every one), that of his 16-cell stage is *in*correct; and unless he again reversed the poles in passing from the 4-cell to the 16-cell stage, his 4-*cell stage is also wrongly oriented.* This being so, the two larger cells of the 4-cell stage are really anterior in the Clavelina studied by Seeliger, just as in the one studied by Van Beneden et Julin.

Another point of difference between the authors mentioned, appears in their discussion of the 8-cell stage. Seeliger states that the larger cells belong to the ventral (ectodermal) half of the egg, whereas Van Beneden et Julin state that they constitute the dorsal (endodermal) half.

I shall presently show that Van Beneden et Julin were mistaken in their determination of the dorsal and ventral faces of the 32-cell and earlier stages. It follows that the larger cells, which they called *dorsal*, were really *ventral*, as stated by Seeliger for the form studied by him. If this is so, another point of difference is removed, for in both the species of Clavelina that have been studied the larger cells of the 8-cell stage are ventral with reference to the axes of the future larva.

In any extended work on cell lineage it is desirable to have some system of naming the individual cells which will indicate readily the exact history of each, — from what part of the matured ovum it has been derived, by how many divisions it is removed from the ovum, and from what other cells these divisions have separated it.

What system one adopts is a matter of choice, but not of indifference. Chabry has employed a fairly good one in his work on Ascidiella. It is too cumbersome, however, for advanced stages, and limited in its applicability. Van Beneden et Julin have simply employed numerals, which give no information whatever as to the derivation of cells. Seeliger has followed no system at all beyond the 16-cell stage, except that of arrows joining cells of the same parentage, which serves to mark the lineage for only one generation.

So far as I know, only one system capable of general application to different types of cleavage has been proposed, that introduced by Kofoid ('94) in his recent work on Limax. As this seems to me to embody several distinct advantages over other systems, I shall follow its general features in this paper.

1. Each cell will be designated by a letter with two exponents.

2. The letter indicates the quadrant of the egg from which the cell in question has been derived, or, in other words, that cell of the 4-cell stage from which it is descended. Viewing the egg from the *ventral* or *ectodermal* side, the left anterior quadrant is A, the right anterior B, the right posterior C, and the left posterior D. In dorsal views, right and left are of course reversed.

As the third cleavage plane is equatorial and separates a ventral from a dorsal hemisphere, I shall designate the cells of the former by capitals and those of the latter by small letters.

3. The first exponent indicates the *generation* to which a cell belongs; that is, the number of cell divisions by which it is removed from the unsegmented ovum.

The ovum is generation one, the 2-cell stage two, the 4-cell stage three, etc.

4. The second exponent indicates the *number* of a cell in a generation, the cells of each quadrant being numbered independently of the other quadrants from the centre of the ventral (*ectodermal*) toward the centre of the dorsal side.* If in any case two cells of common de-

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* In this I do not follow Kofoid, who numbers from the ventral (in his case *endodermal*) toward the dorsal (in his case *ectodermal*) pole. His system of nomenclature I have treated throughout as being, for my purposes, not an index to homologies between blastomeres, but a convenient method of notation.

scent lie in an equatorial position, that one which is nearer the median plane will be given the lower numeral.

It will be noted that I follow the accepted usage, orienting the egg with reference to the future axes of the embryo, and using the terms dorsal and ventral in this sense. I have regarded the egg as viewed from the ventral rather than the dorsal side, because, as is rightly held, the ventral side corresponds to the *ectodermal* pole.

Below is a table, the first six columns of which show the complete cell lineage of the right half of the egg of Clavelina, Ascidiella, or Ciona through the sixth generation (32-cell stage). The terminology of Van Beneden et Julin for the 32-cell stage is given in the seventh column, for purposes of comparison. In the next column is given, in

Cell Lineage of the egg of Clavelina, Ascidiella, or Ciona, right half only, through the 32-cell stage.						CLAVELINA.				
						Terminology of V. B. et J.	Interpretation of V. B. et J.			My Interpretation.
							Their Terminology.		My Terminology.	
First Gen.	Second Gen	Third Gen.	Fourth Gen.	Fifth Gen.	Sixth Gen.	Sixth Gen.	6th, 7th, and 8th Gen.		6th, 7th, and 8th Gen.	6th & 7th Gen.
$\overline{ABCD}^1$	$\overline{AD}^2$	A^3	$A^{4\cdot1}$	$A^{5\cdot1}$	$A^{6\cdot1}$	XIV		20	$A^{7\cdot1}$	$a^{6\cdot6}$
								14	$A^{7\cdot2}$	$a^{6\cdot5}$
					$A^{6\cdot2}$	1		1	$A^{6\cdot2}$	$A^{6\cdot2}$
				$A^{5\cdot2}$	$A^{6\cdot3}$	XV		21	$A^{7\cdot5}$	$a^{6\cdot8}$
								15	$A^{7\cdot6}$	$a^{6\cdot7}$
					$A^{6\cdot4}$	10		10	$A^{6\cdot4}$	$A^{7\cdot8}$
			$a^{4\cdot2}$	$a^{5\cdot3}$	$a^{6\cdot5}$	2		2	$a^{6\cdot5}$	$A^{7\cdot2}$
					$a^{6\cdot6}$	3		3	$a^{6\cdot6}$	$A^{7\cdot1}$
				$a^{5\cdot4}$	$a^{6\cdot7}$	9		9	$a^{6\cdot7}$	$A^{7\cdot7}$
					$a^{6\cdot8}$	4		4	$a^{6\cdot8}$	$A^{7\cdot5}$
		D^3	$D^{4\cdot1}$	$D^{5\cdot1}$	$D^{6\cdot1}$	XVI	XVI*	22	$D^{8\cdot1}$	$d^{6\cdot8}$
								16	$D^{8\cdot2}$	$d^{6\cdot7}$
							19	19	$D^{7\cdot2}$	$D^{6\cdot3}$
					$D^{6\cdot2}$	XIII		13	$D^{7\cdot3}$	$d^{6\cdot6}$
								18	$D^{7\cdot4}$	$D^{7\cdot4}$
				$D^{5\cdot2}$	$D^{6\cdot3}$	7		7	$D^{6\cdot3}$	$D^{7\cdot2}$
					$D^{6\cdot4}$	VIII		8	$D^{7\cdot7}$	$D^{7\cdot8}$
								17	$D^{7\cdot8}$	$D^{7\cdot7}$
			$d^{4\cdot2}$	$d^{5\cdot3}$	$d^{6\cdot5}$	11		11	$d^{6\cdot5}$	$d^{6\cdot5}$
					$d^{6\cdot6}$	12		12	$d^{6\cdot6}$	$A^{7\cdot6}$
	$[\overline{BC}^2]$	(=left half)		$d^{5\cdot4}$	$d^{6\cdot7}$	6		6	$d^{6\cdot7}$	$D^{7\cdot1}$
					$d^{6\cdot8}$	5		5	$d^{6\cdot8}$	$D^{7\cdot3}$
1-cell stage.	2-cell stage.	4-cell stage.	8-cell stage.	16-cell stage.	32-cell stage.	32-cell stage.	44-cell stage.		44-cell stage.	44-cell stage.

the terminology of Van Beneden et Julin, the cell lineage of the 44-cell stage *as interpreted by them;* and in the succeeding column this interpretation is repeated in terms of the nomenclature adopted by me. Finally, in the last column is given *my own interpretation* of the same stage.

The cell lineage for the left half of the egg would be expressed by substituting, in the table, B, b, for A, a, and C, c, for D, d, where my terminology is used; and where the terminology of Van Beneden et Julin is used, by adding a (′) to each of the numerals.

To ascertain the designation of the mother cell of any particular cell, its *first* exponent must be diminished by one; and its *second* exponent, if an even number, must be divided by two, but if an odd number it must first be increased by one and then divided by two.

In order to determine the daughter cells of a particular cell, simply reverse this process; that is, increase the first exponent by one and double the second exponent. The result will be the name of the daughter cell having an *even* second exponent. To determine the other daughter cell, diminish this second exponent by one. Thus the daughter cells of $a^{5.4}$ are $a^{6.8}$ and $a^{6.7}$.

This system I have applied to the 32-cell stage of Van Beneden et Julin in Figures 7 and 8, reproduced from their Figures 9 and 9a, the orientation being corrected to agree with my interpretation.

Similar views of the same stage of Ciona are given in Figures 3 and 4.

It will be seen that this stage of Ciona corresponds cell for cell with the one of Clavelina figured.

In both, the posterior end is marked on one side (Figs. 3 and 7) by the superficially, if not actually, largest pair of cells in the egg at this stage, while underneath them, best seen from the other face of the egg, is the smallest pair of cells (Figs. 4 and 8). The only thing in the egg of Ciona figured which is not perfectly typical is the position of the cells $a^{6.5}$ and $b^{6.5}$, which in other specimens examined lie anterior quite as often as lateral to their sister cells, $a^{6.6}$ and $b^{6.6}$ respectively.

A nearly median optical section parallel to the sagittal plane of the egg exhibited in Figures 7 and 8 is reproduced in Figure 13, and an actual transverse section of the same stage of Ciona is represented in Figure 15. It will be seen that in both cases the cells of one face, which are apparently very large when viewed from the surface, are really thin and superficial; while the apparently small cells of the other face are really high, columnar, and of considerable volume.

These figures leave no doubt that the view of the Ciona egg given in Figure 3 corresponds with that of the egg of Clavelina given in Figure 7, and that the view of the Ciona egg given in Figure 4 corresponds with the view of the Clavelina egg given in Figure 8. If then it can be shown by a detailed study of later stages that the orientation given for the egg of Ciona in Figures 3 and 4 is the correct one, it follows that the orientation given for the egg of Clavelina in Figures 7 and 8 is also correct, and that the orientation given to this stage by Van Beneden et Julin is consequently wrong; for they call Figure 7 a dorsal, and Figure 8 a ventral view.

An examination of Figure 3 shows that the thin superficially large cells seen in this view of the egg are preparing for division. These, as the lettering indicates, are derived from the four ventrally situated cells of the 8-cell stage, and will be referred to collectively as the *ventral* or *ectodermal* half of the egg. The latter term must not be understood, however, to imply that all the cells derived from this hemisphere become ectoderm, but merely that ectoderm is its principal derivative.

The other half of the egg, composed of high, columnar cells, will be called the *dorsal* or *endodermal* half of the egg, with the same restriction on the term endodermal as has been made for ectodermal.

Figure 4 shows the cells of the endodermal half to be quiescent, while those of the ectodermal half are preparing for division. A similar acceleration in division of the ectodermal over the endodermal half of the egg was to be observed at the 24-cell stage (Figs. 1 and 2). Here the cells of the ectodermal half are seen to have passed into the sixth generation, while the cells of the endodermal half are only in the spindle stage, preparatory to the division which will carry them into that generation.

Figure 5 represents a ventral view of a stage of forty-six cells, and shows accomplished the divisions foreshadowed in Figure 3. All the cells of the ventral half of the egg, except the pair of small posterior cells, have divided either completely or partially, thus passing into the seventh generation. I should add that no one of the divisions indicated is a matter of inference, but spindles have been observed in every instance; where they were not to be seen in the specimen figured, they have been observed in other specimens not quite so advanced in development.

Figure 6 gives a view of the opposite face of the same egg. The cells of the dorsal half are here seen to be in the sixth generation, and still quiescent. In a single cell, $b^{6.5}$, a pair of astral radiations it visible. This fact does not, however, foreshadow an *immediate* division of the cell, as these radiations often exist some time before the division actually occurs. It does indicate, however, as the sequel will show, that this cell with its mate, $a^{6.5}$, will be among the earliest cells of the endodermal half of the egg to divide.

In Figures 9 and 10 are reproduced Van Beneden et Julin's figures of the 44-cell stage of Clavelina (their Figs. 10 and 10a). The lettering on the *left* half of Figure 9 and the *right* half of Figure 10 indicates what I believe to be the correct interpretation of the cell lineage of this stage. The interpretation of Van Beneden et Julin is indicated (in terms of my own nomenclature) on the *right* half of Figure 9 and the *left* half of Figure 10.*

It will be seen that at this stage, according to my interpretation, exactly the same divisions, with one exception, have occurred in the egg of Clavelina as in the Ciona egg shown in Figures 5 and 6. The exception mentioned is in the case of the most anterior cell of the ectodermal half, on each side of the median plane ($A^{6.2}$ and its mate, Figs. 8 and 9). This cell has not yet divided, but has remained in the sixth generation, whereas in Ciona it has given rise to $A^{7.3}$ and $A^{7.4}$.

Not only has division occurred in corresponding cells in the egg of Clavelina and that of Ciona, but this division has taken place in the same direction in every instance.

There is, however, in the two eggs, a slight difference in the arrangement of the cells resulting from this division, due perhaps to a difference in the order of division. In the egg of Clavelina (Fig. 9, left half), the cells $A^{7.5}$ and $D^{7.3}$ have been pushed toward the median plane so that they are in contact with their mates in the other half of the egg, and separate $A^{7.1}$ from $D^{7.1}$; but in the egg of Ciona (Fig. 5) $A^{7.5}$ and $D^{7.3}$ do *not* reach the median plane, so that $A^{7.1}$ is left in contact with $D^{7.1}$.

Van Beneden et Julin identify the face of the egg represented in Figure 9 with that seen in Figure 8, and that in Figure 10 with the one seen in Figure 7. In doing so they are forced into several very strange correlations. For example, the small posterior cell $C^{6.3}$ of Figure 8 is identified with the rather large cell $D^{7.2}$ of Figure 9; while the real $C^{6.3}$ ($D^{7.2}$ of Fig. 10, left half) is derived from $D^{6.1}$ (Fig. 7), and its sister cell is supposed to have divided again, so that the other derivatives of $D^{6.1}$ ($D^{8.1}$ and $D^{8.2}$ of Fig. 10, left half) are in the *eighth* generation.

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* It will facilitate a comparison with the lettering of the original, if the reader will make use of the table on page 204.

Finally, the nearly median optical section of this egg, reproduced in Figure 14, shows at a glance the absurdity of Van Beneden et Julin's interpretation. Here the relatively large columnar cells $d^{6.7}$, $d^{6.8}$, $a^{6.6}$, and $a^{6.5}$ of my interpretation,* are derived by them from the thin superficial cells $D^{6.1}$ and $A^{6.1}$ of the preceding stage (Fig. 13), the first two together with $D^{6.3}$ as a result of *two successive divisions* of the single cell $D^{6.1}$. On the other hand the small cubical cells $D^{7.1}$, $D^{7.3}$, $A^{7.5}$, $A^{7.1}$, and $A^{7.2}$ are taken by them to represent, *undiminished by division*, the voluminous columnar cells $d^{6.7}$, $d^{6.8}$, $a^{6.8}$, $a^{6.6}$, and $a^{6.5}$ respectively, of the preceding stage.

It is thus seen from an examination of Van Beneden et Julin's own figures, that they have reversed the dorsal and ventral relations in passing from the 32-cell to the 44-cell stage, and have identified the endodermal half of one with the ectodermal half of the other. This fact has been overlooked by all subsequent writers on tunicate embryology, for all have accepted as correct the results obtained by Van Beneden et Julin.

The question next arises, Is the orientation given by Van Beneden et Julin for the 32-cell stage, or that given for the 44-cell stage, the correct one? A strong presumption that the latter is the case exists in the close correspondence in form and size between the endoderm cells of the gastrula figured by them (their Fig. 11d) and the columnar dorsal cells of their 44-cell stage. This presumption becomes as strong as anything except direct observation of Clavelina can make it, when one follows the cell lineage of Ciona through to a stage which marks the beginning of gastrulation, such as I have represented in Figures 11 and 12.

In order to avoid the extension of this paper to an undesirable length, I have not figured the stages intervening between the 46-cell stage and gastrulation. A detailed study of these stages, however, allows me to speak with confidence of the changes which have occurred.

In the 32-cell stage (Figs. 3 and 4), all the cells of both hemispheres were seen to be in the sixth generation, though, as has been stated, the cells of the dorsal half had divided a little later than those of the ventral half. These dorsal cells now remained quiescent, while all the cells of the ventral or ectodermal half, except the small posterior cells $C^{6.3}$ and $D^{6.3}$, divided, and thus brought about the 46-cell stage. Before the division (Figs. 3 and 4), it will be noted, the cells of the ectodermal half were so arranged that twelve of them were marginal and in contact with cells of the endodermal half, while four ($A^{6.1}$, $B^{6.1}$, $C^{6.1}$, and $D^{6.1}$, Fig. 3) were entirely surrounded by cells of the ectodermal half. These four now divide along planes perpendicular to the sagittal plane, so that both the daughter cells of each still abut on the median plane. All the marginal cells, however, except the small posterior pair, which remains quiescent, divide in such a way that only one of the daughter cells of each is still marginal and in contact with cells of the endodermal half (Fig. 5, $A^{7.4}$, $A^{7.8}$, $A^{7.6}$, $D^{7.4}$, $D^{7.8}$, and the corresponding cells in the right half of the figure).

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* See Explanation of Plates, Figure 14.

The next division again involves those cells of the ectodermal half of the egg which are marginal, including this time the small posterior cells $C^{6.3}$ and $D^{6.3}$, which have lagged one generation behind the other cells of the ectodermal hemisphere, but now give off toward the median plane each a peculiar flattened cell of minute dimensions, undoubtedly the "petites cellules cunéiformes" of Van Beneden et Julin. This division also passes through two other cells of the ectodermal half, $C^{7.7}$ and $D^{7.7}$, and six anterior and marginal cells of the endodermal half, namely, $a^{6.5}$, $a^{6.7}$, $d^{6.5}$, and their mates $b^{6.5}$, $b^{6.7}$, and $c^{6.5}$.

Concomitantly with this division of marginal cells, and to a greater or less extent in consequence of it, the endodermal face of the egg becomes flat, its cells being crowded together at their superficial ends and expanding club-shaped beneath the surface. The ectodermal face at the same time becomes more convex, its cells growing thinner and spreading out so as to cover a greater area. The marginal cells gradually extend around on to the endodermal face, and thus gastrulation begins.

At this stage (66-cell) the ectodermal half of the egg consists of forty-four cells, of which twenty-four, the more marginal ones, are in the *eighth* generation, and the remaining twenty, centrally or posteriorly situated, are in the *seventh* generation.

The endodermal half of the egg, on account of its less rapid cleavage, is composed of fewer cells, viz. twenty-two, just half the number in the ectodermal portion. Of these, twelve — lying anterior and marginal — are in the *seventh* generation, and ten — central or posterior — in the *sixth* generation.

It will thus be seen (1) that the cells of the dorsal half have lagged just one generation behind those of the ventral half, and (2) that cell division has been more active in the anterior and lateral marginal portions of the egg. As a consequence of the first mentioned fact, a gastrula is formed with a dorsal blastopore by a process "intermediate between epibole and invagination"; and because of the second, the blastopore closes from the sides and anterior end, so that it ultimately becomes pear-shaped and lies in the posterior half of the embryo.

The part which particular cells take in forming the organs of the larva is reserved for discussion in a later paper, as that part of my work is still incomplete. One thing may, however, be stated positively. The entire endoderm and mesoderm are *not* derived as stated by Van Beneden et Julin from the twelve cells of the 44-cell stage, $a^{6.5}$, $a^{6.6}$, $a^{6.7}$, $a^{6.8}$, $d^{6.7}$, $d^{6.8}$, $b^{6.5}$, $b^{6.6}$, $b^{6.7}$, $b^{6.8}$, $c^{6.7}$, and $c^{6.8}$.

CONCLUSIONS.

1. The conflicting statements of Van Beneden et Julin on the one hand, and Seeliger on the other, regarding the segmentation of the ascidian egg, are explained by the detection of a fundamental error in the work of each.

Seeliger has determined the dorsal and ventral sides of the egg correctly, but reversed the anterior and posterior ends in all his figures of the early stages. Van Beneden et Julin have determined correctly the anterior and posterior ends, but have reversed dorsal and ventral in all stages previous to the 44-cell stage. These errors are clearly indicated by a critical examination of the authors' own figures, and a careful study of the cell lineage of Ciona leaves no doubt of their existence.

2. The correction of Van Beneden et Julin's unfortunate change of orientation necessitates fundamental changes in their conclusions.

(*a*) It is stated by them that the four smaller cells of the 8-cell stage give rise to ectoderm only, while the four larger cells produce both endoderm and ectoderm. On the contrary, neither the four smaller cells nor the four larger ones produce *ectoderm* exclusively; but it is the four larger, not the four smaller ones, which give rise to the greater portion, perhaps all, of the ectoderm.

(*b*) I cannot at present assent to the statement of Van Beneden et Julin that the separation of the germ layers is complete at the 44-cell stage.

3. In *Ciona*, from the sixth to the eighth generations at least, cell multiplication is *more* rapid in the anterior and lateral portions of the egg, and this fact is an important element in determining the shape and position of the gastrula mouth.

CAMBRIDGE, May 9, 1894.

POSTSCRIPT.

Since the foregoing was written I have had the opportunity of making further observations upon the living egg of Ciona immediately after fertilization and during the early stages of segmentation. I have repeatedly seen the polar globules and observed continuously the cleavage stages following their formation. These observations lead to the surprising but unavoidable conclusion, that the point on the surface of the egg at which the polar globules form becomes later the centre of the *dorsal* or *endodermal* half of the egg. The evidence for this conclusion will be presented and its significance discussed in a future paper.

September 29, 1894.

POSTSCRIPT No. 2.

After the preceding was already in print, Paul Samassa's recent paper was received (Zur Kenntniss der Furchung bei den Ascidien. Arch. f. mik. Anat., Bd. XLIV. Heft I. pp. 1–14, Taf. I. und II. Ausgegeben 15 Sept., 1894).

Samassa's conclusions agree in a gratifying manner with my own, that Van Beneden et Julin on the one hand, and Seeliger on the other, were mistaken in their orientation of the Ascidian egg. Here, however, the agreement ends, for my own interpretation of the cell lineage differs radically from that of Samassa.

In the earlier part of this paper I have pointed out how fatal to the conclusions of Van Beneden et Julin was their *conjecture* as to the manner of division in only three pairs of cells in the egg of Clavelina. I cannot forbear saying that a more apt illustration of the utter untrustworthiness of this method of determining cell lineage could not have been offered than is found in Samassa's paper, where the method of conjecture seems to have been followed in a wholesale and entirely unjustifiable manner.

In his account of the 32-cell stage Samassa makes $D^{6.1}$ (his c 11) the sister cell of $D^{6.3}$ (his c 12) both in Ciona and in Clavelina. In doing so he contradicts, though without giving a particle of evidence in support of his views, the recorded observations of Van Beneden et Julin on Clavelina, and of Chabry on Ascidiella, and this too in the face of the fact that Van Beneden et Julin offer the absolutely incontrovertible evidence of karyokinetic figures in support of their interpretation.

In the living egg of Ciona I have myself repeatedly seen the cell divisions leading to the 32-cell stage take place, and my observations agree perfectly with those of Van Beneden et Julin on Clavelina, and of Chabry on Ascidiella, viz. that the cell $D^{6.1}$ (Samassa's c 11) is the sister cell of $D^{6.2}$ (Samassa's c 21) and $D^{6.3}$ (c 12) that of $D^{6.4}$ (c 22). If Samassa expects his statement to stand against all these independent observations, he must present some evidence in its support.

After discussing the 48-cell stage of Ciona, Samassa says: "Die weiteren Theilungen habe ich nicht Zelle für Zelle verfolgt, da dies für die Lösung der mich interessirinder Frage nicht von Bedeutung ist." Yet, again without a particle of evidence and confessedly without detailed observations, he calmly proceeds to declare the *ancestry* and *fate* of the individual blastomeres of subsequent stages! The history *cell for cell* of the cleavage subsequent to the 48-cell stage is *precisely* the *critical* point in deciding the question with which Samassa is concerned, viz. the origin of the endoderm, and in stopping where he did he has stopped short of solving his problem. Worse than this, by his guesses at the cell lineage he has fallen into positive error. For example, the cells 8 and 9 of his Figure 10, which he says are *ectoderm*, are not so at all, but are *mesoderm*. They with their mates in the left half of the egg ultimately form the greater part, perhaps all, of the longitudinal musculature of the tail, being derived from cells corresponding to $D^{8.16}$ and $d^{6.6}$ of my Figure 12. There is no mistaking the identity of these cells; the large size of the nucleus, which Samassa has observed for his cell 8, and would have found to hold good at a slightly different stage for 9 also, is one of their distinguishing characteristics; their peculiar stainability under proper treatment is another. Samassa assures us that these cells are derived from the ventral or ectodermal cells of the 8-cell stage, and represent, with a few cells posterior to them, "die erste Anlage der Medullarwülste." With these statements I squarely take issue. Of those under consideration, only the cell 8 (my $D^{8.16}$) with its mate in the left half of the egg, is derived from the ventral cells of the 8-cell stage, the other cell (9) is derived from the dorsal cells of the 8-cell stage; and *both* 8 and 9 form, not *ectoderm*, but *mesoderm*, being invaginated with the endoderm cells at gastrulation.

In view of these facts, and others which need not be presented at this time, I am forced to take issue with Samassa's main conclusion, "dass bei Ciona und Clavelina durch die dritte Furchung die Trennung der beiden primären Keimblätter erfolgt." For Ciona at least this is *not* true, and the close correspondence which Samassa himself has recognized between the early stages of Ciona and those of Clavelina renders its occurrence in Clavelina *exceedingly improbable.*

Davidoff's belief that the first equatorial cleavage separated the two primary germ layers can have no weight one way or the other in deciding the question, for Davidoff did not profess to have traced cell by cell the history of the cleavage subsequent to the 8-cell stage.

If my statements in this Postscript seem to the reader to be as dogmatic as Samassa's, I can only say that they rest, not on confessed inference or conjecture as to the cell lineage, but on repeated observations made on *dozens* of embryos, only *continuous observations of the living egg* or *the presence of karyokinetic figures* in preserved specimens being admitted as sufficient evidence of the genetic relationships of cells. The evidence afforded by my figures will be presented, and a more exhaustive discussion of the subject made, in a subsequent paper.

October 5, 1894.

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EXPLANATION OF PLATES.

Arrows joining cells indicate divisions that have occurred since the last stage figured or described.

A = Anterior. P = Posterior. R = Right. L = Left. D = Dorsal. V = Ventral. For the system of lettering employed to indicate cell lineage, consult the text, pp. 203, 204.

PLATE I.

Ciona intestinalis.

All the figures of this plate are drawn to the same magnification, 400 diameters.

Figure 1. Egg of Ciona, 24-cell stage, ventral view. The cells of the ventral half of the egg number sixteen, and are all in the sixth generation. The most recent division is seen to have been that resulting in the posterior cells $D^{6.3}$, $D^{6.4}$, and $C^{6.3}$, $C^{6.4}$.

" 2. The same egg, dorsal view. The cells of the dorsal half of the egg are still in the fifth generation, and consequently number only eight. They are, however, about to divide, and the spindles indicate the directions in which divisions will occur.

" 3. Egg of Ciona, 32-cell stage, ventral view. (Divisions which were already completed at the 24-cell stage are again indicated by arrows, for convenience in comparison.)

" 4. The same egg, dorsal view.

" 5. Egg of Ciona, 46-cell stage, ventral view. All the cells of the ventral half of the egg are seen to have divided more or less completely since the 32-cell stage, except the small posterior cells $C^{6.3}$ and $D^{6.3}$.

" 6. The same egg, dorsal view. All the cells of the dorsal half of the egg are still in the sixth generation, as in Figure 4.

Fig. 1.

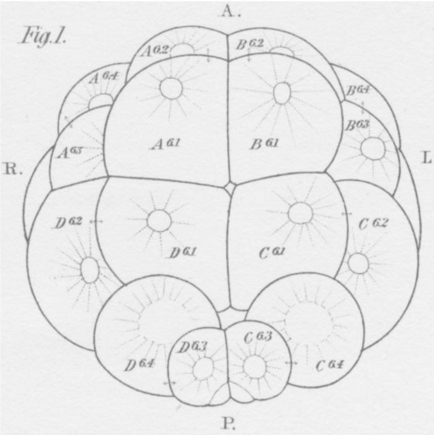


Fig. 2.

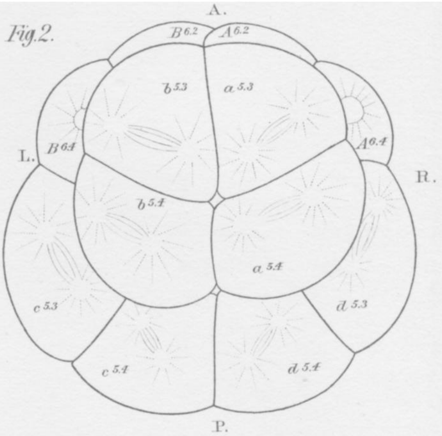


Fig. 3.

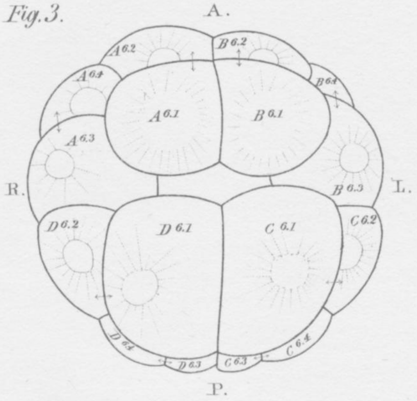
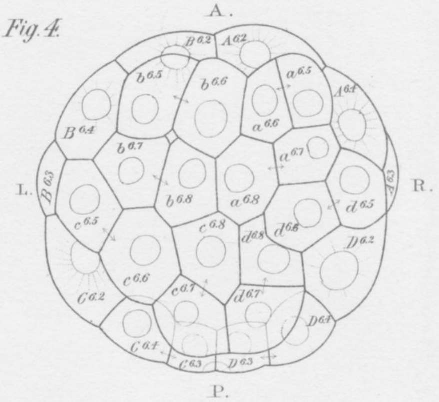


Fig. 4.



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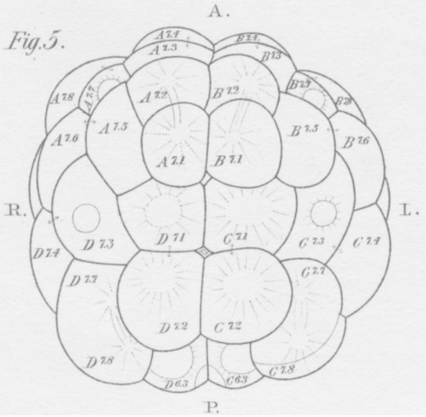
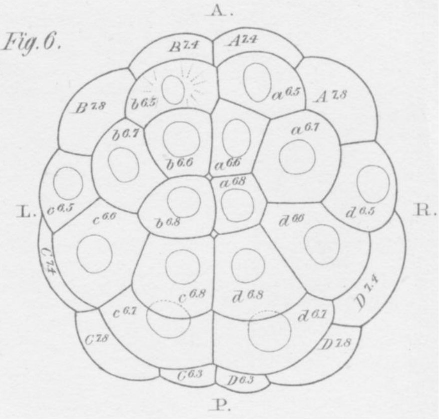


Fig. 6.



W.E.C. del.

B. Meisel, lith. Boston.

PLATE II.

Figures 7, 8, 9, 10, 13, and 14 are copied from Van Beneden et Julin's figures of *Clavelina Rissoana*. The others are of *Ciona*; magnification, 400 diameters.

Figure 7. Egg of Clavelina, 32-cell stage, with corrected orientation, ventral view. (After Van Beneden et Julin's Fig. 9a).

" 8. The same egg, dorsal view. (After Van Beneden et Julin's Fig. 9.)

" 9. Egg of Clavelina, 44-cell stage, ventral view. (After Van Beneden et Julin's Fig. 10.) On the *left* side of the figure is given the corrected cell lineage, on the *right* side, the cell lineage according to Van Beneden et Julin.

" 10. The same egg, dorsal view. (After Van Beneden et Julin's Fig. 10a.) On the *right* side of the figure is given the corrected cell lineage, on the *left*, the cell lineage according to Van Beneden et Julin.

" 11. Egg of Ciona, 66-cell stage, ventral view. Only the marginal cells of the ventral half have divided since the stage shown in Figures 5 and 6.

" 12. The same egg, dorsal view. By more rapid division in the ventral half of the egg certain of its marginal cells have been shoved around on to this face of the egg. Six marginal and anterior cells of the dorsal half have also divided since the 46-cell stage.

" 13. A nearly median optical section, parallel to the sagittal plane, of the egg of Clavelina shown in Figures 7 and 8, with corrected orientation. (After Van Beneden et Julin's Fig. 9c.)

" 14. A nearly median optical section, parallel to the sagittal plane, of the egg of Clavelina shown in Figures 9 and 10. (After Van Benenen et Julin's Fig. 10c.)

" 15. An actual transverse section of an egg of Ciona in the 32-cell stage

Fig. 7.

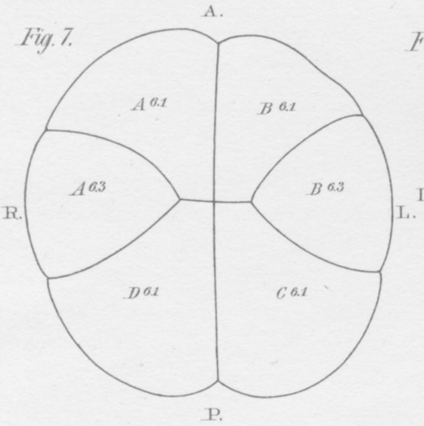


Fig. 8.

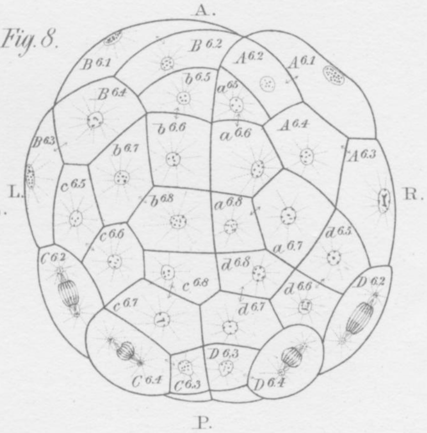


Fig. 13.

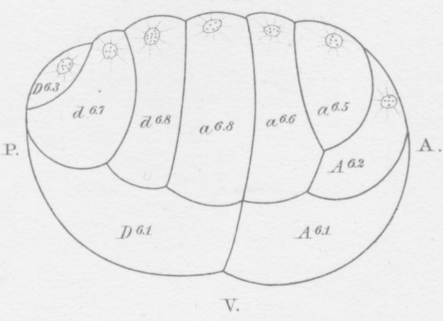


Fig. 9.

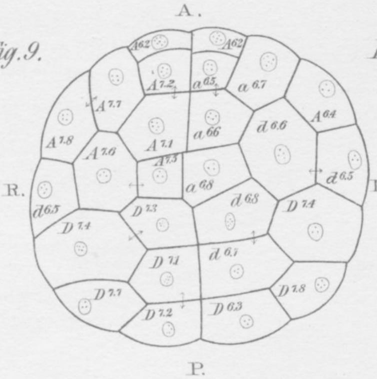


Fig. 10.

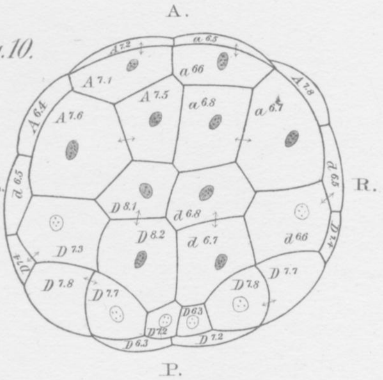


Fig. 14.

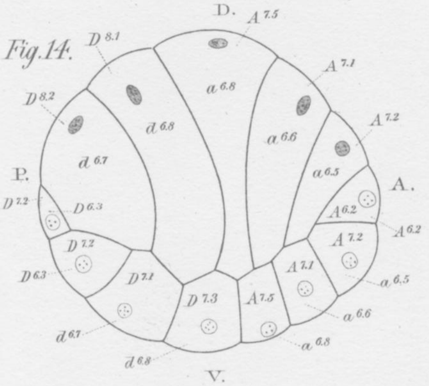


Fig. 11.

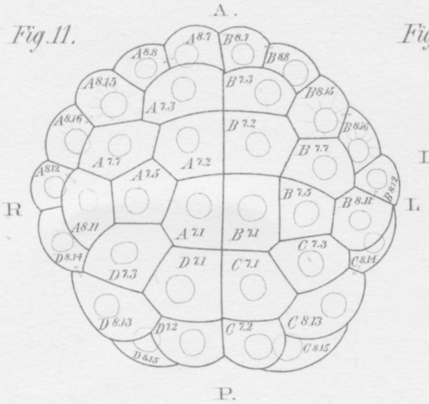


Fig. 12.

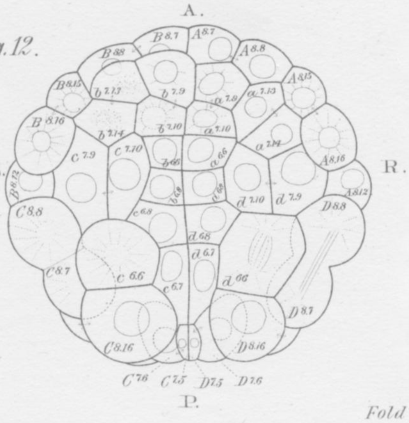
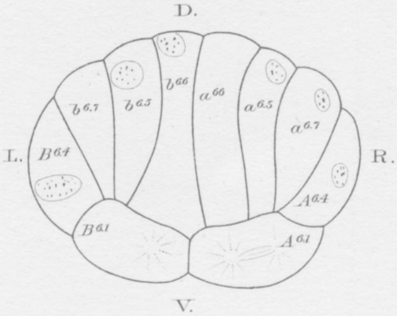


Fig. 15.



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